CLAIMS

1. A method for detecting any one of multiple chromosomal disorders in a single assay, which method comprises the steps of:

a. making a polymerase chain reaction (PCR) mixture by mixing in a vessel components comprising:

(i) eukaryotic genomic DNA;

(ii) a plurality of pairs of forward and reverse DNA primer oligonucleotides wherein one primer of each said pair is complementary to a 3′ sequence of a targeted segment of a first DNA strand of the eukaryotic DNA and the other primer is complementary to the 3′ sequence of the second strand of the targeted segment, the length of the segment of eukaryotic DNA being between about 50 and about 300 base pairs, wherein one of the primers of each pair has a detectable label attached to its 5′ end, and wherein a plurality of the pairs of primers are each targeted to a segment of a selected different chromosome of interest which is indicative of a potential chromosomal disorder and one pair is targeted for a segment of a control gene; and

(iii) PCR buffers and enzymes necessary to carry out PCR amplification;

b. conducting a PCR for between about 5 and about 60 temperature cycles to create amplified PCR products;

c. purifying said products of step (b) and obtaining single-stranded DNA having the detectable labels,

d. contacting a microarray with products of step (c), which microarray has a plurality of spots that each contain DNA oligonucleotide probes having nucleotide sequences complementary to a nucleotide sequence of one of said strands of each of said targeted segments;

e. hybridizing said DNA oligonucleotide probes and said PCR-amplified label-containing single-stranded products;

f. detecting the presence and relative quantity of the PCR-amplified products hybridized to the microarray by imaging the microarray; and

g. diagnosing whether or not a chromosomal disorder exists with respect to one or more of said selected different chromosomes by comparing said imaging of the relevant spots on said microarray for each said targeted segment of a selected chromosome to the imaging of spots relevant to said control gene and then to results obtained from similar testing of genomic DNA known to be normal.

2. The method according to claim 1 wherein said step of diagnosing includes the application of rule-based algorithms to the detection results of step (f) prior to final comparison to said results for normal genomic DNA of the same gender.

3. The method according to claim 1 wherein at least two of the targeted segments of eukaryotic genomic DNA selected are associated with potential microdeletions of chromosomal DNA that would give rise to chromosomal disorders selected from the group consisting of:

Williams-Beuren syndrome,
Cri du chat syndrome, and
DiGeorge syndrome.

4. The method according to claim 1 wherein at least two of the targeted segments are selected to detect chromosomal aberrations selected from the group consisting of trisomy 13, trisomy 18, trisomy 21 and X- and Y-chromosome anomaly.

5. The method according to claim 1 wherein said detectable labels are color-detectable labels.

6. The method according to claim 5 wherein said color-detectable labels are attached to the reverse primers and the forward primer of each pair has phosphate at its 5′ end.

7. The method of claim 6 wherein said detectable labels are fluorescent dyes.

8. The method of claim 1 wherein the double-stranded product of step (b) is first purified and then the sense strands of the purified product are digested with an exonuclease to obtain the single-stranded labeled antisense strand in step (c).

9. The method of claim 1 wherein the control gene is GAPD.

10. The method of claim 1 wherein the sizes of the probes range from about 25 to about 60 nucleotides and the targeted segments are between about 100 and 200 base pairs long.

11. The method of claim 1 wherein two microarrays are used in parallel and the imaging results from both are compared as an initial check on the validity of the hybridizing and imaging steps.

12. A kit to detect chromosomal disorders, which kit comprises:

(a) a plurality of pairs of DNA oligonucleotides that will function as primers in a polymerase chain reaction (PCR) for amplifying mammalian genomic DNA, wherein one primer of each pair of oligonucleotides is complementary to a 3′ nucleotide sequence of a first strand of a targeted segment of mammalian genomic DNA and the other primer of each pair of oligonucelotides is complementary to the 3′ nucleotide sequence of the second strand of the targeted DNA segment, one said primer of each pair having a detectable label covalently linked to the 5′ end thereof;

a plurality of said pairs of DNA primers being targeted to amplify segments of selected different chromosomes of interest which are indicative of potential chromosomal disorders, and one said pair being targeted to amplify a segment of a control gene;

(b) buffers and enzymes for carrying out (i) a PCR, (ii) DNA – DNA hybridization and washing, and (iii) colorimetric quantitation;

(c) at least one microarray having a plurality of spots, at least one of which spots has attached thereto DNA sequences complementary to the label-bearing amplified strand of each respective targeted genomic DNA segment; and

(d) means for diagnosis for chromosomal disorders using intensity imaging results from hybridization reactions between PCR amplification products and the respective spots on the microarray, which means utilizes imaging results from similar testing of normal genomic DNA.

13. The kit according to claim 12 wherein a pair of duplicate microarrays are provided and rule-based algorithms are provided as a part of said diagnosis means for analyzing the results of said intensity scanning of said microarray.

14. The kit according to claim 12 wherein at least two of said pairs of primers are targeted to segments of mammalian genomic DNA wherein there is a potential for a microdeletion of chromosomal DNA which would give rise to chromosomal disorders selected from the group consisting of:

Williams-Beuren syndrome,
Cri du chat syndrome, and
DiGeorge syndrome.

15. The kit according to claim 12 wherein said pairs of primers are targeted to at least two DNA segments selected to detect chromosomal aberrations selected from the group consisting of trisomy 13, trisomy 18, trisomy 21 and X- and Y-chromosome anomaly.

16. The kit according to claim 12 wherein said one primer of each pair has a fluorescent dye label and said other primer of each pair has phosphate at its 5′ end.

17. The kit of claim 12 wherein said one pair of primers target GAPD as the control gene.

18. The kit according to claim 12 wherein the sizes of the probes range from about 25 to about 60 nucleotides and the targeted DNA segments are between about 120 and 200 base pairs long.

19. The kit according to claim 12 wherein said enzymes include an exonuclease for digesting the sense strand of the PCR-amplified product.

20. A method for detecting any one of multiple chromosomal disorders in a single assay, which method comprises the steps of:

a. making a polymerase chain reaction (PCR) mixture by mixing in a vessel components comprising:

(i) eukaryotic genomic DNA;

(ii) a plurality of pairs of forward and reverse DNA primer oligonucleotides wherein one primer of each said pair is complementary to a 3′ sequence of a targeted segment of a first eukaryotic DNA strand and the other primer is complementary to the 3′ sequence of the second strand of the target segment, the length of the segment of eukaryotic DNA being between about 100 and about 250 base pairs, wherein one of the primers of each pair has a color-detectable label attached at the 5′ end thereof, and wherein a plurality of the pairs of primers are targeted to segments of selected different chromosomes of interest which are indicative of potential chromosomal disorders and one pair is targeted for a segment of a control gene; and

(iii) PCR buffers and enzymes necessary to carry out PCR amplification;

b. conducting a PCR for between about 5 and about 60 temperature cycles to create amplified PCR products;

c. purifying said products of step (b) and obtaining single-stranded DNA having the color-detectable labels by digestion of one strand of the amplified double-stranded PCR product,

d. contacting a microarray with products of step (c), which microarray has a plurality of spots that each contain DNA oligonucleotide probes having nucleotide sequences complementary to a nucleotide sequence of one of said strands of said targeted segments;

e. hybridizing said DNA oligonucleotide probes and said PCR-amplified label-containing single-stranded products;

f. detecting the presence and relative quantity of the PCR-amplified products hybridized to the microarray by colorimetric imaging of the microarray; and

g. diagnosing whether or not a chromosomal disorder exists with respect to one or more of said different chromosomes of interest by first comparing said imaging of a relevant spot on said microarray for each said chromosome of interest to the imaging of a spot relevant to said control gene to obtain an I- ratio; then comparing each I-ratio to N-ratios that have been obtained as a result of similar testing of genomic DNA known to be normal.

21. The method according to claim 20 wherein said I-ratios are subjected to rule-based algorithms that involves the adjustment of each I-ratio prior to its use in final diagnosis by using an average C-factor which is obtained after first comparing all of the I-ratios with the respective N-ratios to obtain individual C-factors.